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COMPOSITION AND METHOD FOR REDUCING TOXICITY OF BIOLOGICALLY-ACTIVE FACTORS

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Cross-Reference to Related Application

The present application is a continuation-in-part application of pending prior application Serial No. 08/033,385, filed March 18, 1993, and entitled "Method for Reducing Toxicity of Biologically-Active Factors."

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Technical Field

The present invention relates to compositions and methods for reducing the toxicity of biologically-active factors, such as cytokines and growth factors. In addition, the present invention comprises vaccines which are effective in immunizing a human or animal against a biologically-active factor while reducing or eliminating the toxicity of the factor. More particularly, the present invention relates to compositions comprising a colloidal metal in combination with a biologically-active factor which renders the biologically-active factor non-toxic.

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Background of the Invention

Various biologically active compounds have been isolated from humans or animals which have been reported to have therapeutic efficacy. These compounds include cytokines and growth factors. However, it has been found that when these

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various factors are isolated and purified and then injected into a human or animal, they often cause severe side effects and exhibit unwanted toxicity. Because of this toxicity, it has been difficult to use the compounds therapeutically. In addition, it has been
5 difficult to use the active compounds as antigens to produce antibodies against the molecules.

Aluminum compounds have been used to form water-insoluble antigenic substances. For example, U.S. Patent No. 3,577,523, issued to *Stolar, et al.*, discloses the combination
10 of aluminum tannate with antigenic extracts to form water-insoluble slow release antigenic substances. More generally, the *Stolar, et al.* patent discloses the use of antigenic depot agents incorporating water-insoluble antigenic substances that slowly release active agents that are absorbed without adverse systemic
15 reactions or other adverse side effects.

Metals have also been used in capsular polysaccharide metal complex vaccines. For example, in U.S. Patent No. 4,740,589, issued to *Moreno*, a bacterial capsular polysaccharide constituent was complexed with a metal, preferably aluminum or
20 ruthenium, for the prophylaxis and treatment of bacterial diseases. This patent also discloses the formulation of a three component complex which contains a polysaccharide, a metal, and a third constituent of bacterial outer-membrane protein. The '589 patent discloses that the complex contains a weight
25 percentage of lipopolysaccharide "insufficient to produce significant toxic effects", the weight percentage being generally 1% or less. Finally, the disclosure in the '589 patent application is limited to the use of the complexes for prophylaxis and treatment of bacterial diseases.

U.S. Patent No. 3,269,912, issued to *Grase*, discloses a depot vaccine comprising a finely divided aluminum oxide, either aluminum oxide or aluminum oxide aerosol having had
30 absorbed thereon at least one antigen derived from a virus, bacteria, or ectotoxoid, dispersed in an aqueous medium. The '912 patent also discloses that the vaccine forms a colloidal
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dispersion of the individual spherical crystals of aluminum oxide in the solution.

Selected metals have also been used as components of stable adjuvant emulsion compositions. It is known in the art that aluminum, as the monostearate, or in the form of hydrated salts of fatty acids, are emulsifying agents, or stabilizers of the emulsion in the vaccine composition.

However, substantial need exists for a therapeutically effective composition with reduced toxicity, that may be used in therapies for a wide range of immune diseases, cancers, viral diseases and bacterial diseases. In addition, there is a need for a composition that can reduce the toxicity of normally toxic biologically-active compositions so that the compounds can be used as vaccines in the human or animal.

Summary of the Invention

The present invention satisfies the above-described needs by providing effective compositions containing normally toxic compounds that, when in combination with a colloidal metal, result in significantly reduced toxic side effects. Generally, the composition of the present invention comprises an admixture of a colloidal metal, such as gold chloride (HAuCl_4) in combination with a substance which normally is toxic to a human or animal capable of producing an immune response, wherein the composition when administered to a human or animal is less or non-toxic.

The method of use of the above composition comprises administering to a human or animal an effective amount of a composition comprising a colloidal metal, such as HAuCl_4 , in combination with a substance which normally is toxic to a human or animal capable of producing an immune response, wherein the composition, when administered to a human or animal, is less toxic or non-toxic.

The use of colloidal metals in combination with normally toxic biologically active compounds may be used in

cancer and immune disease therapies. Current therapies which consist of administering factors such as interleukins to a human or animal are marginally effective but produce significant, toxic side effects. Also, the toxic side effects limit the amount of biologically-active factors that may be administered, and therefore limits the efficacy of the therapy. Additionally, some otherwise therapeutic compounds are not used at all due to their toxicity. The combination of a colloidal metal with such biologically-active factors reduces toxicity while maintaining the therapeutic effectiveness.

Accordingly, it is an object of the present invention to provide a composition that is capable of reducing the toxicity of biologically-active factors.

A further object of the present invention is to provide a composition containing higher concentrations of biologically-active factors than are currently utilized due to the toxicity of the substances.

Another object of the present invention is to provide a composition to be used in cancer therapies which results in reduced side effects from toxicity.

Yet another object of the present invention is to provide a composition for immune disease therapy which results in reduced side effects from toxicity.

Another object of the present invention is to provide a therapeutic method for immune disease therapy which results in reduced toxic side effects and maintains its beneficial effects.

Yet another object of the present invention is to provide a method of vaccinating a human or animal against a normally toxic biologically-active factor..

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the preferred embodiment.

Brief Description Of The Drawings

Figure 1 illustrates the serum antibody titers in mice immunized with murine IL-6 combined with colloidal gold.

5 Figure 2 illustrates the retention of biologic activity of IL-1 after treatment with gold.

Figure 3 illustrates the efficiency with which gold binds IL-6.

Detailed Description

10 The terms "toxic reaction," and "toxicity," as used herein, include, but are not limited to, the following responses of an animal or human: fever; edema, including cerebral edema; psychosis; autoimmune diseases; hemorrhage; shock, including hemorrhagic shock; sepsis; cachexia; or death. The term
15 "colloidal metal", as used herein, includes any water-insoluble metal particle or metallic compound dispersed in liquid water (a hydrosol). The term "biologically-active factors" includes, but is not limited to, Interleukin-2 ("IL-2"), lipid A, phospholipase A2, endotoxins, staphylococcal enterotoxin B and other toxins, Type I
20 Interferon, Type II Interferon, Tumor Necrosis Factor, IL-1, IL-6, IL-8, IL-4, Transforming Growth Factor-B, Lymphotoxin, IL-5, Migration Inhibition Factor, IL-3, Granulocyte-Macrophage Colony-Stimulating Factor ("CSF"), Monocyte-Macrophage CSF, Granulocyte CSF, IL-7, IL-10, IL-11, IL-12, IL-13, vascular
25 epithelial growth factor ("VEGF"), Angiogenin, transforming growth factor ("TGF α "), heat shock proteins, carbohydrate moieties of blood groups, Rh factors, fibroblast growth factor, and other inflammatory and immune regulatory proteins.

30 The present invention comprises a composition and method for administering normally toxic biologically-active factors to a human or animal. Generally, the composition according to the present invention comprises an admixture of a colloidal metal in combination with a substance which normally is toxic to a human or animal capable of producing an immune
35 response, wherein the composition, when administered to a

human or animal, is less or non-toxic to the human or animal. The composition optionally includes a pharmaceutically-acceptable carrier, such as an aqueous solution, or excipients, buffers, antigen stabilizers, or sterilized carriers. Also, oils, such as paraffin oil, may optionally be included in the composition.

The composition of the present invention can be used to vaccinate a human or animal against biologically-active factors which are normally toxic when injected. In addition, the present invention can be used to treat certain diseases with cytokines or growth factors. By admixing the biologically-active factors with the colloidal metal before administering them to the human or animal, the toxicity of the biologically-active factor is reduced or eliminated thereby allowing the factor to exert its therapeutic effect.

Current therapies which comprise administering biologically-active factors to a human or animal are somewhat effective yet produce significant toxic side effects. Further, the toxic side effects limit the amount of antigen that may be administered, and therefore limit the efficacy of the therapy. Additionally, the toxicity of some biologically-active factors precludes their use in such therapies. The combination of a colloidal metal with such biologically-active factors reduces toxicity while maintaining or increasing the therapeutic results thereby improving the efficacy as higher concentrations of biologically-active factors may be administered, or by allowing the use of combinations of biologically-active factors. The use of colloidal metals in combination with biologically-active factors therefore allows the use of higher concentrations of biologically-active factors or formerly unusable toxic substances, to be administered to humans or animals.

The term "biologically-active factors" includes, but is not limited to, Interleukin-2 ("IL-2"), lipid A, phospholipase A2, endotoxins, staphylococcal enterotoxin B and other toxins, Type I Interferon, Type II Interferon, Tumor Necrosis Factor, IL-1, IL-

6, IL-8, IL-4, Transforming Growth Factor-B, Lymphotoxin, IL-5, Migration Inhibition Factor, IL-3, Granulocyte-Macrophage Colony-Stimulating Factor ("CSF"), Monocyte-Macrophage CSF, Granulocyte CSF, IL-7, IL-10, IL-11, IL-12, IL-13, VEGF, Angiogenin, TGF α , heat shock proteins, carbohydrate moieties of blood groups, Rh factors, fibroblast growth factor, and other inflammatory and immune regulatory proteins.

The colloidal metal may for example be selected from the metals in groups IIA, IB, IIB and IIIB of the periodic table, as well as the transition metals, especially those of group VIII. Preferred metals include gold, silver, aluminum, ruthenium, zinc, iron, nickel and calcium. Other suitable metals may also include the following in all of their various oxidation states: lithium, sodium, magnesium, potassium, scandium, titanium, vanadium, chromium, manganese, cobalt, copper, gallium, strontium, niobium, molybdenum, palladium, indium, tin, tungsten, rhenium, platinum, and gadolinium. The metals are preferably provided in ionic form, (preferably derived from an appropriate metal compound) for example the Al $^{3+}$, Ru $^{3+}$, Zn $^{2+}$, Fe $^{3+}$, Ni $^{2+}$ and Ca $^{2+}$ ions. A preferred metal is gold, particularly in the form of Au $^{3+}$. An especially preferred form of colloidal gold is HAuCl $_4$ (E-Y Laboratories, Inc., San Mateo, California). Another preferred metal is silver, particularly in a sodium borate buffer, having the concentration of between approximately 0.1% and 0.001%, and most preferably as approximately a 0.01% solution. The color of such a colloidal silver solution is yellow and the colloidal particles range from 1 to 40 nanometers. Such metal ions may be present in the complex alone or with other inorganic ions.

The amount of colloidal metal that is used in the present invention is between approximately 0.001 mg/ml and 1.0 mg/ml with the more preferred amount of colloidal metal being between approximately 0.01 mg/ml and 0.1 mg/ml. The amount of the composition according to the present invention to be administered to humans or animals varies according to the disease

to be treated, the biologically-active factor or factors used in the therapy, the species involved, and the physical state of the individual to be treated.

5 To prepare a vaccine against biologically-active factors, the preparation of the selected biologically-active factor is admixed with the colloidal metal in a salt-free medium, preferably deionized water. The salt-free medium may optionally be buffered with, for example, Tris buffer. In one embodiment
10 of the invention, the colloidal metal solution is diluted 1:1 with the solution of biologically-active factors.

The medium should preferably not contain sodium ions. A colloidal gold solution has a light pink color, this color should not change when adding the solution containing the biologically-active factors. If the colloidal gold solution turns
15 from pink to purple, this indicates that the gold has precipitated and cannot be reconstituted for effective immunization. The shelf-life of an admixture of colloidal gold and biologically active factor(s) is approximately 24 hours.

The admixture of biologically-active factors and colloidal metal is then injected into an appropriate animal. For example, rabbits weighing between approximately two to five kilograms suffered no noticeable side-effects after they were administered, every two weeks, a composition comprising
20 colloidal gold and 1 mg of cytokine, either IL-1 or IL-2. Because the biologically-active factor is not toxic when administered according to the present invention, the optimal quantity of antigen can be administered to the animal. The compositions according to the present invention may be administered in a single dose or they may be administered in multiple doses, spaced over a suitable
25 time scale to fully utilize the secondary immunization response. For example, antibody titers have been maintained by administering boosters once a month.

30 The vaccine may further comprise a pharmaceutically acceptable adjuvant, including, but not limited to Freund's complete adjuvant, Freund's incomplete adjuvant,
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lipopolysaccharide, monophosphoryl lipid A, muramyl dipeptide, liposomes containing lipid A, alum, muramyl tripeptide-phosphatidylethanolamine, keyhole and limpet hemocyanin. A preferred adjuvant is Freund's incomplete adjuvant, which preferably is diluted 1:1 with the mixture of colloidal metal and biologically-active factor.

The method of use of the composition comprises administering to a human or animal an effective amount of the composition comprising a colloidal metal admixed with a biologically-active factor or factors, wherein the composition when administered to a human or animal, is less or non-toxic. The composition according to the present invention can be administered as a vaccine against a normally toxic substance or can be a therapeutic agent wherein the toxicity of the normally toxic agent is reduced thereby allowing the administration of higher quantities of the agent over longer periods of time.

In practicing this invention, the process by which the composition is administered is not considered critical. The routes that the composition may be administered according to this invention include, but are not limited to, subcutaneous, intramuscular, intraperitoneal, oral, and intravenous routes. A preferred route of administration is intravenous. Another preferred route of administration is intramuscular.

It is known that Interleukin-2 (IL-2) displays significant therapeutic results in the treatment of renal cancer. However, the toxic side effects result in the death of a significant number of the patients. In contrast, if IL-2 is mixed with colloidal gold, little or no toxicity is observed and a strong immune response occurs. The doses previously used for IL-2 therapy have been on the order of 21×10^6 units of IL-2 per 70 kg man per day (7×10^6 units of IL-2 per 70 kg man TID). One unit equals approximately 50 picograms, 2 units equals approximately 0.1 nanograms, so 20×10^6 units equals 1 milligram. In one embodiment of this invention, the amount of IL-2 that has been given to rabbits is approximately 1 mg per 3 kg rabbit. In effect,

the studies of the effects of the administration of biologically-active factors described herein have included doses of more than 20 times higher than that previously given to humans.

5 In another embodiment, where IL-2 (1 mg per 3 kg animal) was administered to 3 rabbits every third day for a two-week period, all the animals appeared to be clinically sick, and two of the animals died from the apparent toxic effects of the IL-2. When the same dose of IL-2 was combined with colloidal gold and then administered to three rabbits for the same two-week
10 period, no toxicity was observed and a significant antibody response resulted in all three animals. A "positive antibody response" as used herein is defined as a three to fourfold increase in specific antibody reactivity, as determined by direct ELISA, comparing the post-immunization bleed with the pre-immunization bleed. A direct ELISA is done by binding IL-2
15 onto a microtiter plate, and determining the quantity of IgG bound to the IL-2 on the plate, by goat anti-rabbit IgG conjugated to alkaline phosphatase. Therefore, it is thought that the biological effects of the IL-2 remain. As the toxicity effects have
20 been minimized, larger concentrations of IL-2 may be administered if necessary where a larger, more effective immune response is required.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing
25 limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present
30 invention and/or the scope of the appended claims.

Example I

This example demonstrates that colloidal gold neutralizes otherwise toxic substances and allows for an antibody
35 response. When IL-2 (1 mg per 3 kg animal) is administered to

three rabbits every third day for a two-week period, all the animals appear to be clinically sick, and two of the animals died from the apparent toxic effects of the IL-2. When the same dose of IL-2 is combined with colloidal gold and then administered to three rabbits for the same two-week period, no toxicity is observed and a significant antibody response results in all three animals. A positive antibody response is defined as a three to fourfold increase in specific antibody reactivity, as determined by direct ELISA, comparing the post-immunization bleed with the pre-immunization bleed. A direct ELISA is done by binding IL-2 onto a microtiter plate, and determining the quantity of IgG bound to the IL-2 on the plate, by goat anti-rabbit IgG conjugated to alkaline phosphatase.

Example II

This example further demonstrates that colloidal gold neutralizes otherwise toxic substances and allows for an antibody response. Endotoxin or lipid A (25, 50, and 100 μ g per 35 mg mouse) are administered by subcutaneous injection every fourth day over a two-week period. For ten mice, endotoxin is given "neat" and for the remaining ten, the endotoxin is mixed 1:1 with colloidal gold. The injection volume is made up by adding potassium carbonate/sodium citrate buffer, pH 6.5 at a 1:1 dilution. The same protocol is also used where lipid A is the test drug.

The animals are checked at 15, 30, and 60 minutes following each injection, and then hourly and daily. The surviving animals are tested for a specific antibody response to the toxic substance they were injected with, either endotoxin or lipid A. Most of the animals injected with endotoxin or lipid A combined with colloidal gold survived, while those injected with the neat endotoxin or lipid A died during the two-week test period. In addition, those animals that did survive did have an antibody response to the specific toxin as determined by direct ELISA.

Example III

This example illustrates the effect of colloidal gold on cytokine activity *in vivo*. A group of mice are given IL-2 at a dose close to that given to cancer patients undergoing immunotherapy. In previous experiments, 18 μ g of IL-2 tablets given to nude mice reduced implant tumor size, but killed the animals within two weeks. See Paciotti, G.F., and Tamarkin, L., Interleukin 2 Differentially Effects the Proliferation of a Hormone-Dependent and a Hormone-Independent Human Breast Cancer Cell Line *In Vitro* and *In Vivo*, *Anti-Cancer Research*, 8: 1233-1240 (1988), which is hereby incorporated by reference.

The efficacy of gold in a murine model system is tested in the following procedure: a group of mice are treated with IL-2 alone, IL-2 mixed with colloidal gold, colloidal gold alone, or saline solutions delivered through an osmotic minipump. The mice are treated for seven days, after which they are sacrificed and their lymphocytes harvested. The cells are stained for T-cell or B-cell markers using specific murine monoclonal antibodies for flow cytometric analysis. Activated T- and B-cells are determined by assessing T-cell numbers, helper T-cell to suppresser T-cell ratios, activated cellular IL-2 receptor, B-cell numbers, and natural killer cell ("NK") numbers.

The few animals that survived being treated with IL-2 alone showed an increase in the T-cell number and activity (as determined by IL-2 receptors). Virtually all the animals survived IL-2 treatment in combination with colloidal gold, and these animals showed an increase in both B-cell function (as determined by activated B-cells and total IgG, measured by direct ELISA) an increase in T-cell function (as determined by T-cell number, and activity, using IL-2 receptor numbers as an index of activity), and an increase in NK activity.

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Example IV

The following biological experiment shows that colloidal gold reduces the toxicity of lipopolysaccharide (LPS). LPS is the lipid/sugar moiety of bacterial cell walls. When injected into an animal, this molecule mimics many of the clinical responses of septic shock. Thus, mice were injected with varying amounts of LPS in the presence or absence of colloidal gold. Specifically Balb/c mice were injected with either 100 or 400 μg of LPS (strain W.E. coli 055:B5; 10 mg/ml in water; Difco Labs) with or without colloidal gold. The pH of the 15 nm colloidal gold mixture (E.Y. Labs) was adjusted to approximately 10, while the pH of the LPS was adjusted to 8 with 0.1 N NaOH. Subsequently, appropriate volumes (i.e., 10 μl for the 100 μg dose and 40 μl for the 400 μg dose) was then added to 500 μl of colloidal gold. The mixture was allowed to stand for 30 minutes and subsequently injected (i.p.) into the mice.

Within 12 hours after the injections, all mice exhibited clinical signs of depression and anergasia. Within 24 hours after the injection control mice in the 400 μg dose began to die. By 72 hours all of the control mice in the 400 μg dose died while 75% of the gold treated mice were alive and began showing signs of clinical improvement (i.e., movement). Furthermore, although subjective, the mice in the 100 μg dose which were treated with gold were more active throughout the 36 hours of observation.

Example V

The following experiment describes the use of colloidal gold as a putative adjuvant for generating mouse antibodies against murine IL-6. This experiment was performed with two goals in mind: 1) To determine if colloidal gold could be used as an adjuvant in generating an immune response to "self-antigens" (i.e., generating an immune response to a mouse protein using a mouse model); Secondly, since IL-6 is one of the cytokines thought to be involved in cancer cachexia, metastasis

and sepsis, then the ability to generate antibodies in an autologous system may prove advantageous in generating a vaccine to the IL-6 and similar endogenous compounds.

Briefly described, the experiment is as follows.

5 Several mice were immunized with colloidal gold/murine IL-6 mixture as described above. Approximately 3 weeks later, the mice were sacrificed and trunk blood was collected and analyzed for the presence of antibodies to murine IL-6 by a direct ELISA, as described above. The results from the direct ELISA, the
10 determination of the serum antibody titers in mice immunized with murine IL-6 combined with colloidal gold, are illustrated in Figure 1. Figure 1 demonstrates that the mice had generated an antibody response to murine IL-6 thus indicating that the gold may be useful in generating antibodies to endogenous (i.e., self)
15 toxins as well as cytokines thought to be involved in sepsis, cancer cachexia and metastasis.

Example VI

20 The following experiment shows that cytokines mixed with colloidal gold retain their biological activity. The model used for these experiments is one which is well known in the art. See Paciotti, G.F., and L. Tamarkin, *Interleukin 1 directly regulates hormone-dependent human breast cancer cell proliferation in vitro*, Mol Endocrinol., 2: 459-464, 1988; and
25 Paciotti, G.F., and L. Tamarkin, *Interleukin-1 differentially synchronizes estrogen-dependent and estrogen-independent human breast cancer cells in the G₀/G₁-phase of the cell cycle*, AntiCancer Research, 11: 25-32, 1991. The model is based on the ability of the cytokine, IL-1, to directly inhibit the growth of
30 estrogen-responsive human breast cancer cells, MCF-7. Briefly described, IL-1 alone inhibits the growth of these cells through a well-characterized IL-1 receptor on the surface of these breast cancer cells.

35 The following experiment shows the ability of IL-1 when mixed with colloidal gold to retain its biological activity by

determining its ability to inhibit the growth of these cells. Approximately 8,000 MCF-7 cells were plated in 24-well tissue culture plates. On the next day, 15 nm gold particles were centrifuged at 14,000 rpms for 10 minutes and resuspended in sterile water. Human IL-1 α was reconstituted in water to an initial stock of 5×10^{-5} M in water. The pH of the gold and IL-1 was adjusted to approximately 8.0 with 0.1 M NaOH. Prior to mixing, the IL-1 was diluted to a working stock of 2×10^{-6} M, 2×10^{-8} M, and 2×10^{-10} M, which contained 250 μ l of the gold (final volume = 0.5 ml). Gold controls consisted of 250 μ l of gold and 250 μ l of sterile water. Subsequently, each working stock was further diluted 1/20 in tissue media resulting in final concentrations of 10^{-7} , 10^{-9} , and 10^{-11} M. These solutions along with the appropriate controls were then added directly to the MCF-7 cells. The data presented in Figure 2 are the number of cells present at various days after the addition of IL-1 with or without the gold.

Example VII

The following experiment shows the efficiency with which gold binds cytokine. This experiment demonstrates that the protein is removed from the solution when it is combined with gold and then centrifuged. The experiment used the IL-6 standard from ARI's CytokitTM-6. Prior to mixing, the pH of the gold and cytokine solutions were adjusted to pH 9 with 0.1 N NaOH. This protein was either preincubated with gold or water prior to using it in ARI's diagnostic kit for IL-6. Following this incubation, the colloidal gold/IL-6 mixture was centrifuged and the supernatants were used to generate a standard curve. As can be seen from Figure 3 the gold was very effective at binding virtually all the IL-6 in the dose-range of the assay, removing the IL-6 from the supernatant. Even at the highest final concentration (1000 ng/ml) of IL-6, the gold removed approximately 90% of the IL-6 in the solutions. This amount is

based on the OD of the 1000 ng/ml IL-6/gold supernatant, which is similar to the 100 ng/ml IL-6 standard alone.

Example VIII

5 The following experiment shows the physical changes
in the gold colloid solution upon its mixing with IL-6, a potential
antigen for a vaccine. Although the gold particles are
approximately 15 nm in size, they cannot be filtered through a
0.22 μ m syringe filter. We attribute this to the nature of the gold
10 particles in this colloid mixture. It is theorized that the gold as a
colloidal mixture forms aggregates larger than the individual
spheres. Although the individual particles are smaller than the
pore size of the filter, the aggregates are much larger and thus
are not filterable. However, we observed that once the colloidal
15 gold is incubated with protein it easily filters through the 0.22 μ m
filter. Thus, the binding of a cytokine appears to change the
physical interactions of the gold particles with each other; making
the gold particles act as single 15 nm particles and enabling the
particles to be readily filtered. This experiment defines the
20 nature of the binding of an antigen to the colloidal metal.

It should be understood, of course, that the foregoing
relates only to specific examples of the present invention and that
numerous modifications or alterations may be made therein
25 without departing from the spirit and the scope of the invention as
set forth in the appended claims.